be directly involved in the pathogenesis of PD; indeed, extensive accumulation of nitrated aS has been detected in amyloid aggregates in PD post-mortem brain tissue. In vitro, oxidative modifications to aS can inhibit fibrillation and lead to the build-up of stable oligomers, which may cause increased toxicity. Here, we use single molecule fluorescence techniques (fluorescence correlation spectroscopy and single molecule Förster energy transfer) to investigate the influence of oxidative modifications on the molecular mechanisms of aS aggregation and membrane interaction. aS is unstructured in solution but residues 1-90 form an alpha-helix upon binding lipid bilayers. Tyrosine nitration leads to decreased binding of aS to lipid vesicles, which might entail a loss of aS native function. Interestingly, we find that nitration of tyrosines located at the C-terminus of the protein, which stays unstructured upon membrane binding, can modulate the affinity of the N-terminus. Another consequence of nitrative insult to the protein is the formation of di- and oligomeric aS species by di-tyrosine cross-linking. We find that protein cross-linking does not perturb the protein's ability to form an alpha-helix upon membrane binding, although the binding affinity is altered. Nitrative stress has been implicated to be involved in PD pathology and the characterization of its effects on aS conformation and membrane interaction will help to refine our understanding of the toxic form(s) of aS.

**1349-Pos**
**Nature of the Low pH Alpha Synuclein Conformational State Revealed with Single Molecule Fluorescence**
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Alpha-Synuclein (αS) is a natively unstructured protein that is strongly implicated in Parkinson's disease (PD) pathogenesis. Aggregated αS is the main component of Lewy body plaques, a hallmark of PD, but smaller αS oligomers are thought to be the cytotoxic agent responsible for neuronal death in the disease. Thus, understanding how this monomeric, unstructured protein becomes a toxic oligomeric state is a vital question in understanding the role of αS in PD. Low pH has been shown to induce the formation of a partially folded structure in αS, which is likely the first step in αS aggregation pathways. We use single molecule Förster resonance energy transfer (smFRET) and fluorescence correlation spectroscopy (FCS) to study a low pH αS conformational state. smFRET measurements have shown that C-terminal residue 130 makes close contact with the central, hydrophobic region of αS at low pH. The N-terminal helix-forming region of αS undergoes little change from neutral to low pH. We have also used guanidine denaturation experiments monitored by smFRET to study the stability of the low pH state. Characterizing the nature of the low pH αS state is critical for understanding this transition, as therapeutic targeting of this state could stop the aggregation process before it even begins.

**1350-Pos**
**Monitoring the Lipid- Binding Properties of Beta- and Gamma- Synuclein using Fluorescence Correlation Spectroscopy (FCS)**
**Vanessa C. Ducas**, Elizabeth Rhoades.
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The synucleins are a family of natively unstructured proteins consisting of α-, β-, and γ-synuclein, which are primarily expressed in neurons. The synucleins have been linked to the pathogenesis of various neurological disorders, such as Parkinson's disease (α-synuclein) and Dementia with Lewy bodies (α- and β-synuclein). Interestingly, β-synuclein might also have a protective role in neurodegenerative diseases that are associated with formation of α-synuclein aggregates. γ-synuclein was first identified in breast cancer cells, and was later found to be overexpressed in other types of cancer, such as ovarian cancer and retinoblastoma. Recent studies indicated that overexpression of γ-synuclein promotes cancer cell survival and metastasis. Still, the biological relevance of the synucleins is yet to be elucidated. All the synucleins share a 6-residue motif, KTKEGV, in their N-terminal region that is commonly found in lipid-binding proteins (apolipoproteins), and it is thought that their native function likely entails binding to biomembranes. In this study, we use fluorescence correlation spectroscopy (FCS) to monitor the lipid- binding properties of β- synuclein and γ-synuclein. Our findings will help determine the underlying factors governing the synuclein- membrane interactions, as well as the strength of these interactions, which would not only reflect the native functions of these proteins, but would also help understand their involvement in disease states.

**1351-Pos**
**Determining the the Effects of Disorder on Binding Affinity**
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It has long been believed that proteins require a defined three-dimensional structure to perform their specific functions. However, a class of proteins called intrinsically disordered proteins has been identified that do not require a stable structure to perform their functions. These proteins play important roles in many diverse biological processes including signal transduction, transcription, and cell division. Therefore, understanding how these proteins recognize and bind to other proteins to perform their functions is an important question. FlgM is an 88-residue intrinsically disordered protein from bacteria that regulates flagella synthesis by binding the RNA transcription factor Sigma 28. When FlgM is bound to Sigma 28, it inhibits transcription of the genes encoding the late flagella proteins. The FlgM protein is an interesting IDP since FlgM genes from different bacteria exhibit different degree of disorder region. Specifically, our lab has shown that the FlgM gene from A. aeolicus is significantly more ordered than the S. typhimurium FlgM. It is predicted that the more ordered the protein, the higher the affinity of the FlgM for Sigma 28. We are using a combination of Isothermal Titration Calorimetry (ITC) and fluorescence to determine the equilibrium binding constant and the binding kinetics for FlgM binding to Sigma 28 using proteins from a series of different bacteria, including A. aeolicus, S. typhimurium, E. coli, P. aeruginosa, and B. subtilis.

**1352-Pos**
**Mechanism of Small-Molecule Binding by Intrinsically Disordered Proteins**
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We have demonstrated multiple examples of small molecules that are capable of specific binding to relatively short segments of intrinsically disordered (ID) proteins. Molecules that bind to the ID monomer of the cMyc bHLHZip protein are capable of disrupting the extensive protein-protein interface normally formed between cMyc and its heterodimerzation partner Max. The kinetics of the disruption is dependent on the location of the small-molecule binding site along the bHLHZip structure. One site allows rapid disruption with the small molecule acting as a wedge while two other sites are inaccessible to inhibitors when cMyc is dimerized and function only by trapping cMyc when it is in the dissociated, monomeric state. High-affinity, bivalent inhibitors retain the fast disruption profile of one of the constituent parts.

## Ribosomes & Translation

**1353-Pos**
**Simulations of the Ribosome Suggest Reversible Transitions and Parallel Pathways are Involved in the Large-Scale Functional Motions of tRNA During Translation**
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Through model building and large-scale computer simulations, we present a structural framework for understanding the molecular mechanisms of transfer RNA (tRNA) motion through the ribosome. In the context of tRNA accommodation (the process by which tRNA enters the ribosomal complex), these models predict that highly-specific functional motions are determined by the atomic details of the ribosome. Significant findings include 1) large-scale reversible fluctuations in tRNA position precede complete tRNA accommodation, 2) the accommodation process possesses multiple kinetic intermediates that may be related to ribosomal ''proofreading'' and 3) parallel pathways of accommodation may allow incoming tRNA molecules to be re-routed in response to changes in cellular conditions. In addition to illuminating the role of the ribosome's structure, this work also predicts that large changes in entropy in the individual tRNA molecules lead to energetically favorable accommodation pathways. The dynamics predicted in these models are validated through comparison with crystallographic data, explicit-solvent simulations and smFRET experiments.

**1354-Pos**
**Fast Biosynthesis of GFP Molecules - A Single Molecule Fluorescence Study**
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Numerous studies showed that protein folding and maturation can differ substantially between *de novo* synthesized proteins and *in vitro* refolded proteins.